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Effects of aminoguanidine on pre- and post-irradiation regional cerebral blood flow, systemic blood pressure and plasma histamine levels in the primate [1]

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Abstract

Exposure to ionizing radiation causes hypotension, cerebral ischemia and release of histamine (HA). To investigate the relationship among these three responses, rhesus monkeys (*Macaca mulatta*) received aminoguanidine (AG) (1 mg/kg), then were given either 50 Gy whole-body irradiation or sham-irradiation. Monkeys receiving AG had lower mean arterial blood pressure (MABP) than saline-treated controls. Compared to controls, rCBF was lower in irradiated monkeys but pre-treatment with AG did not influence this effect. Among untreated, irradiated monkeys, HA levels were increased only at two minutes post-irradiation, but among AG-treated, irradiated monkeys, HA levels were higher at all times postirradiation. Radiation-induced release of HA may be associated with radiation-induced hypotension and reduced rCBF, but failure of AG to alter rCBF suggests that released HA may not be the sole mediator of these effects. Because elevations in plasma HA are probably due to HA derived from degranulation of mast cells, release of other bioactive substances from mast cells may also influence these cardiovascular effects. Surprisingly, in sham-irradiated monkeys, AG alone had a slight but significant hypotensive effect.

Introduction

Studies have shown elevated levels of circulating blood histamine (HA) in humans undergoing radiation therapy [1] and increased levels of HA in plasma of nonhuman primates [2–5] following irradiation. HA is implicated in radiation-induced hypotension [2] and in postirradiation reduced cerebral blood flow [3, 4]. Antihistamines attenuated the effects of HA released after irradiation and the concomitant early transient incapacitation (ETI) in the monkey [5, 6]. ETI, the complete, transient cessation of motor performance, occurs within the first 30 min after exposure to supralethal doses of ionizing radiation [7].

The metabolic pathways of HA in nonhuman primates have been studied less extensively than those in humans. In primates, as in rodents, HA is methylated, forming *tele*-methylhistamine (t-MH), which in turn is oxidized to *tele*-methylimidazoleacetic acid. HA is also deaminated by diamine oxidase (DAO), forming imidazoleacetaldehyde, which is converted to imidazoleacetic acid [8–10]. The pattern of labeled HA metabolites recovered in urine following slow infusion of small amounts of labeled HA into healthy humans suggests that, under these conditions, the contributions of each

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pathways to HA degradation are nearly equal [11]. Oral administration of 1 mg/kg of aminoguanidine (AG) (1 mg/kg) nearly abolished the amounts of oxidative metabolites, and enhanced the quantities of methylated metabolites and unmetabolized HA recovered in urine [11].

To facilitate measurements of HA in the plasma of rhesus monkeys, DAO is often inhibited, because its concentration in plasma is much higher in this species than in most other animals [12]. For example, one group (using a method with less sensitivity to HA than the one described below) reported that levels of HA in plasma from rhesus monkeys ranged from nondetectable to about 0.2 ng/ml [13]. Therefore, it is often useful to use a carbonyl reagent, such as AG, to inhibit enzymatic degradation of HA. Also, such inhibition would reduce the rates at which HA is metabolized after its levels are elevated [8, 14]. Although AG facilitated the measurement of HA levels in plasma, this agent had little effect on the hypotension of irradiation [unpublished data]. The effect of AG on cerebral blood flow in monkeys has not been previously investigated.

Because a relationship probably exists between release of HA, reduced mean arterial blood pressure (MABP), and decreased regional cerebral blood flow (rCBF) following irradiation [3, 4], we hypothesized that AG would alter these radiation-induced physiological responses. To test this hypothesis, we investigated the effects of AG treatment on these three parameters in irradiated and sham-irradiated monkeys.

Materials and methods

In this study, we used 23 rhesus monkeys (*Macaca mulatta*), weighing between 2.3 kg and 4.9 kg (3.3 ± 0.1 SEM). The animals were divided randomly into four groups: (1) six given saline (i.v.) 60 minutes before sham-irradiation, (2) five given AG (10 mg/kg, i.v.) in saline 60 minutes before sham-irradiation, (3) six given saline (i.v.) 60 minutes before irradiation, and (4) six given aminoguanidine (10 mg/kg, i.v.) in saline 60 minutes before irradiation. Food was withheld from all animals for 18 hours before the experiment, but water was available *ad libitum*. Research was conducted according to the principles enunciated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council (USA). Monkeys were initially anesthetized in their cages with ketamine hydrochloride (20 mg/kg, i.m.) supplemented with 0.015 mg/kg atropine sulfate, and were then moved to the laboratory for the remainder of the experiment.

A systemic venous catheter was used to administer physiological saline and the principal anesthetic, α-Chloralose (100 mg), and supplemental infusions were provided as needed, based on heart rate, blood pressure, respiration rate, blood pH, and peripheral reflexes. A femoral arterial catheter was used to withdraw blood for blood chemistry and blood gas determinations and to measure systemic arterial blood pressure.

Approximately 2 hours before irradiation or sham-irradiation, the animals were intubated with a cuffed endotracheal tube and ventilated using a forced volume respirator to maintain stable blood pH and oxygen tension. After insertion of the endotracheal tube, each animal was placed on a circulating water blanket to maintain body temperature between 36 °C and 38 °C. A rectal probe monitored body temperature.

Using a technique previously described [3, 4, 15, 16], platinum-iridium wire electrodes were placed in the left and right hippocampi (CA1 region) in order to measure rCBF by hydrogen clearance. Measurements were taken for 30 minutes before irradiation or sham-irradiation and for 60 minutes thereafter. This technique is essentially an amperometric method that has been successfully employed in similar studies [3, 4, 15, 16].

After recording for 30 minutes, the animals were disconnected from the respirator and recording apparatus and irradiated in a separate room, using a bilateral, whole-body exposure to gamma ray photons from a ^{60}Co source located at the Armed Forces Radiobiology Research Institute. Exposure was limited to a mean dose of 46.8 seconds at 62.4 Gy/minutes steady state, free-in-air. Dose-rate measurements at depth were made with an ionization chamber placed in a tissue equivalent model. The measured midline tissue dose rate was 58.0 Gy/minutes, producing a calculated total dose of 50 Gy, taking into account the rise and fall of the radiation source. (The Gray (Gy), the Systeme International (SI) unit for absorbed dose, corresponds to an energy absorption of 1 J/kg or 100 rad.)

The animals were reconnected to the respirator and recording apparatus at 4 minutes postirradia-

tion or sham-irradiation and measurements were continued for a minimum of 60 minutes. At 30 and 10 minutes before irradiation or sham-irradiation, and at 2, 4 and 6 minutes after irradiation or sham-irradiation, blood samples were taken via the arterial catheter to determine plasma HA levels. Blood samples were taken to monitor stability of blood pH and oxygen tension, and respiration was adjusted to maintain pre-irradiation levels. MABP was determined via the arterial catheter during the experiment. After the experiment, the animals were humanely euthanized with an i.v. injection of saturated $MgSO_4$ while still under anesthesia. Brains were removed and dissected for visual verification of electrode placement.

Blood samples were drawn from the arterial catheter with plastic syringes and transferred to prelabeled, chilled collection tubes containing EDTA. The blood was then centrifuged (5 °C) and the plasma was transferred to polypropylene tubes, rapidly frozen, and stored at −80 °C until analyzed.

For measurements of plasma levels of HA, plasma was thawed, and 300 µl were transferred to 400-µl polypropylene tubes containing 100 µl of 100 m*M* sodium phosphate (NaP) buffer (pH 7.9). The mixture was vortexed, boiled for 10 minutes, cooled, and centrifuged at 50000 × *g* for 20 minutes. The supernatant (25 µl) was analyzed for HA. Quantitation of HA in samples collected 2, 4, or 15 minutes after irradiation often exceeded linear segments of the standard curve for HA content (2.5–250 pg base). Therefore, aliquots from these samples were diluted with 25-m*M* NaP buffer to 400 µl; 25 µl of this diluted mixture were analyzed for HA.

HA was methylated by tritiated S-adenosyl-L-methionine (SAM) in the presence of exogenous histamine N-methyltransferase (HMT) to form tritiated t-MH by modifications (in preparation) of the single isotope method of Salberg et al. [17] such that 2.5 pg (about fmol) of HA could be reliably measured.

Supernatants were transferred to 400-µl polypropylene tubes containing 5 µl of 50-m*M* NaP. HA standard solutions (5 µl of 2.5–250 pg base in 50-m*M* NaP) were added to tubes containing 25 µl of 25-m*M* NaP (pH 7.9). Methylation began after addition of a cocktail (10 µl) containing unlabeled SAM (Sigma Chemicals), [methyl-^{3}H]SAM (New England Nuclear), and HMT prepared from rat kidney, in 50-m*M* NaP buffer (pH 7.9). Final incubation concentrations were 1.03 µ*M* (13.4 Ci/mmol) and 14.8 µg protein (256 µmol/g protein/hr) for SAM and HMT, respectively. Blanks were identical to standard preparations but devoid of HA. To determine HA recovery, 5 µl of a solution containing 25 pg of HA in 50-m*M* NaP were added to aliquots of each assayed sample, and were processed in parallel.

The 40-µl mixture was incubated for 60 minutes in ice water, then quenched with 12.5 µl of 0.4-N $HClO_4$ containing unlabeled t-MH (50 µg/ml). After vortexing, 10 µl of 10-N NaOH and 200 µl of chloroform were added sequentially, vortexed, and centrifuged (2000 × *g*) for 2 minutes. The aqueous layer was aspirated away, and 50 µl of 3-N NaOH was added. The mixture was vortexed, re-centrifuged, and the aqueous layer was removed. Aliquots (100 µl) of the organic phase were transferred to scintillation counting vials. After the chloroform evaporated, scintillant (NEN-963, New England Nuclear) was added, and each vial was counted in a Beckman LS-3801 spectrophotometer. Standards and blanks were determined in quadruplicate, and the unspiked supernatant samples and those used for recovery, were analyzed in triplicate.

Blood pressure and blood flow data were grouped into 10 minute intervals, measured in relation to midtime of radiation. Data from each interval were averaged and plotted at the middle of the interval. The Shapiro-Wilk Test was used to assess normality of values of the various sample groups [18]. The Wilcoxon Rank Sum Test was used for the statistical analysis of the blood pressure, blood flow and HA data. A 95% level of confidence was employed to determine significance. Because all animals were treated identically before irradiation or sham-irradiation, and because the data for control and test animals showed no significant difference among monkeys at 30 minutes and 10 minutes before irradiation, pre-irradiation data for irradiated and sham-irradiated animals were combined for each monkey.

Results

The Shapiro-Wilk test, which assesses the composite hypothesis of normality [18], indicated that data from many samples were sufficiently inconsistent ($p<0.05$) with a normal distribution. This

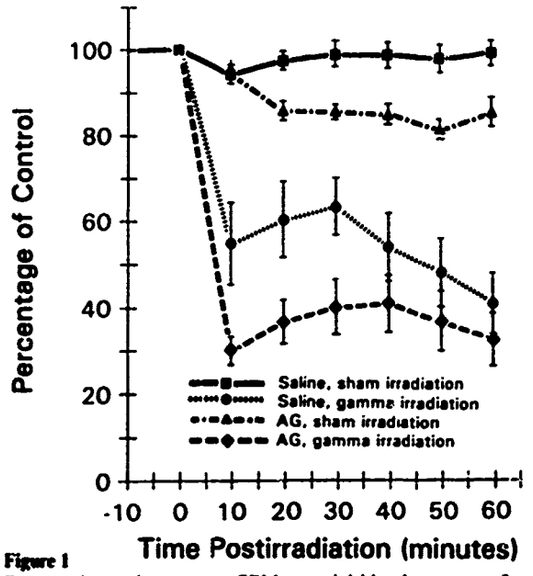

Figure 1
Percent change in mean (±SEM) arterial blood pressure after exposure to 50 Gy, whole-body, gamma irradiation, compared to a calculated pre-irradiation mean (99.8±1.8 mm Hg) determined from observations taken 30 min and 10 min before exposure. Changes in each monkey were based on their individual pre-irradiation mean arterial blood pressure. Twelve monkeys were pre-treated with saline 60 min before sham or gamma irradiation and eleven monkeys were pre-treated with aminoguanidine (AG) (10 mg/Kg, i.v.) 60 min before sham or gamma irradiation.

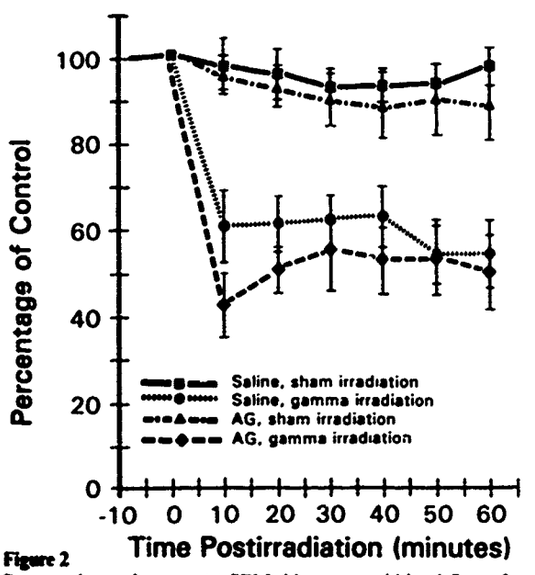

Figure 2
Percent change in mean (±SEM) hippocampal blood flow after exposure to 50 Gy, whole-body, gamma irradiation, compared to a calculated pre-irradiation mean (63.4±3.0 ml/g of tissue/min) determined from observations taken 30 min and 10 min before exposure. Changes in each monkey were based on their individual pre-irradiation hippocampal blood flow. Twelve monkeys were pre-treated with saline 60 min before sham or gamma irradiation and eleven monkeys were pre-treated with aminoguanidine (AG) (10 mg/Kg, i.v.) 60 min before sham or gamma irradiation.

finding encouraged us to use alternate methods, such as distribution-free techniques or non-parametric statistical procedures [18]. Therefore, the Wilcoxon Rank Sum Test was used for the final statistical analysis.

The MABP of both groups of irradiated animals decreased from the pre-irradiation mean of 99.8±1.8 mm Hg within 10 minutes after irradiation (Fig. 1). The irradiated group not treated with AG showed a drop to the 10 minutes postirradiation level that was 52% of the pre-irradiation value. The irradiated group treated with AG showed blood pressure levels that dropped to 30% of the pre-irradiation levels. These two levels were also significantly different for the remaining five observations in the two groups. The two control, sham-irradiated groups (one with AG and one without AG) were significantly different from each other at 20, 30, 50 and 60 minutes after sham-irradiation. However, these two groups were not significantly different from each other after 10 minutes and 40 minutes. The two groups of animals not treated with AG, one irradiated and one sham-irradiated, showed blood pressure levels that were always significantly different from each other after irradiation. Likewise, the two AG-treated groups of animals were significantly different after irradiation. In each of the four groups, the respiration of each subject was maintained at pre-irradiation levels, and the blood gas data revealed a general stability of blood pH and oxygen tension throughout the experiment (data not shown).

Fig. 2 displays a pre-irradiation mean blood flow of 63.4±3.0 ml per 100 g of tissue per minute in the hippocampus. Compared with sham-irradiated monkey, postirradiation blood flow values for both groups of irradiated animals showed a rapid ($p<0.01$) significant decline within 10 minutes after irradiation. The rCBF for the irradiated group treated with AG dropped markedly to 42% of the pre-irradiation level, while for the untreated, irradiated animals it only dropped to 60%. Although at most times after irradiation, rCBF of AG-treated monkeys was less than those given saline.

there was no significant difference between hippocampal blood flow levels of the two irradiated groups at any time after irradiation. Similarly, the two control, sham irradiated groups were not significantly different from each other or from pre-irradiation levels at any time after irradiation. The two groups of animals that were not treated with AG (one irradiated and one sham-irradiated) were significantly different ($p<0.01$) at each postirradiation time. Comparison of the two groups treated with AG also showed a significant difference ($p<0.01$) at all times after irradiation.

The mean (±SEM) pre-irradiation plasma HA level, averaged from 30 minute and 10 minute pre-irradiation levels of all monkeys, was 1.38 ng/ml ± 0.26 ng/ml (Fig. 3). Levels of HA for both sham-irradiated groups of monkeys showed no significant changes at any time during the experiment. After irradiation, HA levels for both irradiated groups showed abrupt increases within 2 minutes to levels that were significantly higher than the pre-irradiation levels. HA levels in Plasma of the AG-treated, irradiated group were also significantly higher than those in the sham-irradiated monkeys and in untreated, irradiated monkeys for all postirradiation observations. However, among monkeys that were not pretreated with AG, plasma levels of HA in irradiated monkeys significantly exceeded plasma levels in sham-irradiated monkeys after 2 minutes ($p<0.01$), but were not significantly higher after 4 or 6 minutes.

For all monkeys ($n=23$), the mean (±SEM) of the HA levels in samples of plasma collected 30 minutes before (−30 minutes) irradiation or sham-irradiation (1.46 ng/ml ± 0.49 ng/ml) exceeded those of samples collected 10 minutes before (−10 minutes) irradiation (1.30 ng/ml ± 0.23 ng/ml). Although this difference was not statistically significant, levels of HA were more often (16 of 23) higher in samples taken at −30 minutes. For all sham-irradiated monkeys ($n=11$), there were no correlations ($p<0.2$) between levels of HA in plasma collected at −30 minutes or at −10 minutes versus levels in samples taken 2, 4, or 6 minutes after sham-irradiation. There was a correlation (Spearman's rho = 0.66, $p<0.05$), however, between samples collected at −30 minutes and −10 minutes. For sham-irradiated monkeys, the mean (±SEM) levels (ng/ml) of HA in plasma collected at −30 minutes and −10 minutes, averaged together (1.66 ± 0.58), slightly but significantly (Wilcoxon test) exceeded

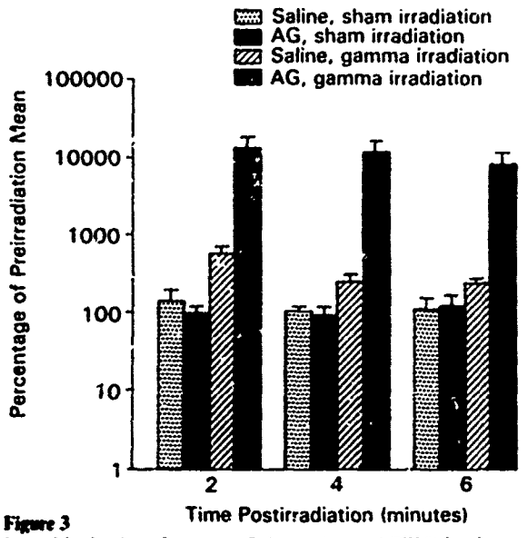

Figure 3
Logarithmic plot of percent of change (mean ± SEM) in plasma HA concentration after exposure to 50 Gy, whole body, gamma irradiation. Differences in concentration were calculated from the mean of levels (1.38 ± 0.26 ng/ml) determined from all monkeys 30 min and 10 min before exposure. Changes in each monkey were based on their individual pre-irradiation levels of HA. Twelve monkeys were pre-treated with saline 60 min before sham or gamma irradiation and eleven monkeys were pre-treated with aminoguanidine (AG) (10 mg Kg, i.v.) 60 min before sham or gamma irradiation.

mean levels of samples collected 2 minutes (1.14 ± 0.34, $p<0.01$), 4 minutes (0.99 ± 0.24, $p<0.05$), or 6 minutes (0.78 ± 0.09, $p<0.05$) after sham-irradiation; however, none of the mean levels from 2, 4, or 6 minutes after sham-irradiation different significantly from any other. No significant differences (each $p<0.3$) existed among sham-irradiated monkeys in levels of HA for drug-treated or drug-free monkeys at any time during the study.

Discussion

The initial precipitous decline in postirradiation rCBF in the rhesus monkey reported here (Fig. 2) has been observed previously [3, 4, 15, 16, 19]. This decline previously has been associated consistently with an immediate fall in MABP. In primates, a critical MABP of 50% to 60% of normal is necessary for adequate autoregulation of cerebral circulation [6, 19, 20].

The measurements of blood flow in the hippocampus of monkeys exposed to 50 Gy of gamma radiation, when plotted at postirradiation times

(Fig. 2), presents a graph strikingly similar to the MABP graph of the monkey (Fig. 1). The abrupt increase in plasma HA levels 2 minutes after irradiation (Fig. 3) coincides with the initial depression in MABP (Fig. 1) and rCBF (Fig. 2). The involvement of HA is further supported by investigators who have reported the attenuation of radiation-induced ETI by the administration of antihistamines [6].

The levels of HA we found corroborate results from previous studies [2–5] by showing an immediate rise within 2 minutes after irradiation in nonhuman primates. Our results also demonstrate that AG has a pronounced effect on the postirradiation levels of HA. Irradiated monkeys given AG had levels of HA in plasma that were at least ten fold higher than those that were not treated with AG (Fig. 3). No significant differences were noted in the plasma HA levels of the two groups of sham-irradiated monkeys at any time. Additionally, the plasma HA levels in the saline-treated, irradiated monkeys were not significantly different from the levels in the control animals 4 minutes and 6 minutes post-irradiation. This is indicative of the primate's rapid metabolism through the oxidative pathway of the extremely high levels of HA in plasma that follow exposure to gamma radiation. Before development of techniques with sufficient sensitivity to measure plasma levels of HA in primates, like the one used here, investigators often pretreated primates with a DAO inhibitor to facilitate measurement of plasma HA. We measured plasma HA levels even in sham-irradiated monkeys that were not treated with AG. It seemed logical that, in primates, further studies of the effects of the radiation-induced release of HA (such as hypotension, induction of other intermediate agents, and central nervous system effects) might show a clearer, more accurate picture without a DAO inhibitor. The presence of a DAO inhibitor clearly affects the metabolism of newly released HA, elevating its peak concentrations and retarding its rate of disappearance (Fig. 3). Thus, using a paradigm that provoked a massive release of endogenous HA, we confirmed the observations of others [21] who showed that AG augmented the levels of HA given to dogs. In dogs and other species, AG shortened survival times following induction of intestinal ischemia [22, 23], presumably by extending the duration of histamine's deleterious effects.

In the absence or presence of a DAO inhibitor, we observed subtle, time-related differences in plasma levels of HA, findings that may extend beyond studies of the effects of radiation. The levels of HA in plasma collected before sham-irradiation of the monkeys showed a general decline with time, from about 1.5 ng/ml (at -30 minutes) to about 0.8 ng/ml in sham-irradiated monkeys. We attribute this small but statistically significant reduction to anesthetic-induced release of HA and its subsequent metabolism. The correlation between levels in samples taken at −30 minutes and −10 minutes, and lack of correlation or significant differences among samples collected after −10 minutes, suggests that the slightly elevated levels of HA may have been declining up until the time of sham-irradiation. At about this time, levels tended to stabilize; random differences among these lower levels probably abolished any relationship between levels in samples collected from the same animals. Conversely, among the same AG-treated, sham-irradiated monkeys, there was a significant relationship between the rank order of levels at −30 minutes and −10 minutes, a sequelae that we anticipated, based on the incomplete metabolism of HA, whose levels were almost certainly elevated after pretreatment with anesthetic. The phenomenon of anesthetic-induced release of HA during the course of surgical manipulation has been seen clinically [24–26]. In addition, the stress of surgery alone has been linked to the release of HA in humans [27]. This experiment shows that without additional doses of anesthetic, such as α-chloralose or atropine (which stimulate release of HA), as much as 2 hours may be required for plasma HA levels to return to near basal levels. This seems to be true whether or not AG is present.

AG pretreatment had a pronounced effect on postirradiation blood pressure. There was a significant difference between the treated and untreated groups of animals 10 minutes after irradiation. This corresponded with the postirradiation levels of plasma HA in the irradiated groups. Therefore, using AG to inhibit the enzymatic deamination of HA caused a significant difference in the postirradiation level of plasma HA and in the postirradiation hypotension. However, changes associated with AG may not be related exclusively to alterations in HA levels in plasma. For example, when comparing the two nonirradiated groups of animals, one can see that treatment with AG alone

had a significant effect on blood pressure (Fig. 1) although there were no significant differences in levels of HA between these groups in the first few minutes of the study (Fig. 3). The reason(s) for this minor, but statistically significant, 10-percent reduction in MABP is unclear. Also, we cannot explain why HA metabolism was altered in these unconscious monkeys more than 10 minutes after sham-irradiation. The simplest hypothesis to account for these changes is that AG, at the doses used, exerts hypotensive effects independent of its influence on HA metabolism in primates.

Unlike the changes observed in HA levels and blood pressure, rCBF responses in the sham-irradiated groups and irradiated groups were not significantly altered by the administration of AG. Although the two irradiated groups were significantly different from the two sham-irradiated groups, the irradiated groups were not significantly different from each other, nor were the sham-irradiated groups significantly different from each other (Fig. 2).

The drug-related differences in the level of HA (Fig. 3) cannot be reconciled immediately with the lack of a significant difference in rCBF (Fig. 2). Several possibilities may account for this. The postirradiation release of HA may not be directly responsible for reductions in rCBF. Instead, another intermediate agent(s) may be involved. For example, it is established that neurotensin (NT) is rapidly released in response to radiation [4]. Rioux et al. [28] showed in rats that the NT-induced release of HA was associated with cerebral edema, and that the increased resistance to blood flow in the brain was attributed to NT-induced release of serotonin (5-HT). Certainly, HA may affect rCBF through its effect on systemic arterial blood pressure. However, if the continued presence of HA produced a significant difference in blood pressure, it would follow that a significant change in cerebral blood flow would occur if HA were the sole regulator of rCBF.

Conversely, the enormous surge in levels of HA suggests that HA may achieve "ceiling-effects" within minutes. Such levels could trigger a sequela of responses that persist long after the removal of HA (by metabolism or diffusion) has occurred. Because correlations could not be made between several intermediate concentrations of HA and degrees of rCBF, our findings cannot resolve these possibilities. Under less severe provocations of HA release, or after administration of HA or HA agonists, HA alone may be a sufficient trigger to alter rCBF [29].

Besides the potential for HA to alter rCBF through its hypotensive actions, HA is also a direct acting cerebral vasodilator when applied *in vitro* or topically [29]. Furthermore, infusing HA into humans resulted in decreased MABP and altered rCBF [30, 31]. However, in another study, similar treatment altered neither MABP nor rCBF [32]. Therefore, the net effect of small concentrations or higher levels (Fig. 3) of HA on rCBF is unclear.

For these reasons, a unitary hypothesis that attempts to explain radiation-induced effects solely through either intra- or extra-cranial histaminergic mechanisms may be misguided. Mast cells, the likely origin of HA after irradiation-induced degranulation [33], will release other bioactive substances along with HA, including leukotrienes, prostaglandins, 5-HT, and heparin. Radiation-induced release of free radicals also influences mast cell degranulation [33, 34]. However, the administration of disodium cromoglycate [15], an efficient hydrated electron scavenger [35], did not decrease the radiation-induced release of HA in nonhuman primates.

This experiment showed that pretreatment of primates with the DAO inhibitor, AG, altered the postirradiation levels of plasma HA and the hypotensive response to radiation, but did not alter postirradiation rCBF response. It also showed that AG alone produced a small but significant hypotensive effect. The experimental conclusions further suggest that the radiation-induced release of HA may not be directly or solely responsible for the radiation-induced decrease in rCBF. Perhaps the radiation-induced release of another intermediate(s), such as serotonin, may better account for the profound reduction in rCBF [36].

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